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HYDROLYTIC DEGRADATION PROFILING OF EZETIMIBE BY HPLC METHOD

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ABSTRACT

Background

Forced degradation profiling is used to facilitate the development of analytical methodology, to gain a better understanding of active pharmaceutical ingredient (API) and drug product (DP) stability and it also part of new drug application (NDA) registration.

Objectives

To develop and validate a novel RP-HPLC method for determination of ezetimibe and forced degradation products and establish its intrinsic stability by forced degradation study.

Methods

Ezetimibe and its forced degradation products were successfully separated on Phenomenex, C18 column (250 × 4.6 mm, 5 µm) by mixture of methanol, water and acetonitrile (44:36:20) at 1 ml/min flow rate within 15 min and detection was performed by photodiode-array detector (PDA) at 248 nm.

Results

Ezetimibe was completely degraded into five degradants within 30 min in 50% methanolic 0.1 N NaOH at 60°C while it was degraded upto 71.3% into three degradants till 120 min in 50% methanolic 0.1N HCl at 60^0 C.

Conclusions

Drug was faster degraded in alkaline conditions than acidic conditions. The validated RP-HPLC method for ezetimibe may be applied for assay, stability-indicating assay, dissolution studies, as well as routine analysis in pharmaceutical industries.

KEYWORDS: Degradation profiling, Ezetimibe & RP-HPLC

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Original Article

INTRODUCTION

Ezetimibe is new lipid-lowering agent which inhibits the absorption of cholesterol from intestine and used in coronary heart diseases. Through glucuronidation ezetimibe forms an active metabolite ezetimibe glucuronide in liver which comes into small intestine via enterohepatic circulation and it is localized at the brush border of small intestine. As result, inhibition the uptake of dietary and biliary cholesterol at the level of enterocytes, this is the lipid lowering effect. Chemically, ezetimibe is 1- (4-fluorophenyl)-3 (R)-[3-(4-fluorophenyl)-3 (S)-hydroxyl propyl]-4 (S)-(4- hydroxyphenyl)-2- azetidione [1] (Figure 1).

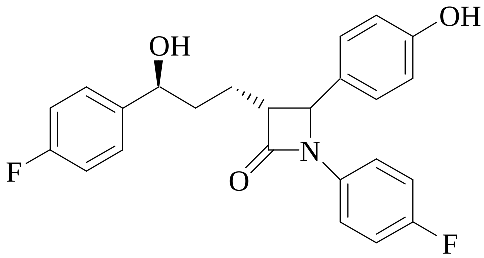


Figure 1: Structure of Ezetimibe

Numbers of chromatographic method were reported for estimation of ezetimibe in tablet dosage from [2], plasma [3], serum [4] and with various drugs such as simvastatin [5], atorvastatin [6], glimperide [7], rosuvastatin [8], valsartan [9]. The present work was aimed to explore intrinsic stability by performing the forced degradation to establish degradation profiling of ezetimibe. As degradation and impurity profiling of active pharmaceutical ingredients (APIs) in pharmaceutical industry have great importance viz. provide data to support identification of possible degradants; degradation pathways and intrinsic stability of the drug molecule; results of onetime forced degradation studies should be included in Phase 3 INDs (Investigational New Drugs); provides information about degradation pathways of API etc [10].

EXPERIMENTAL

Materials

Ezetimibe working standard was obtained as generous gift from Torrent Pharmaceuticals, Baroda. HPLC grade acetonitrile and methanol were obtained from Merck (India) Limited.

Chromatograph

The HPLC system consisted of a solvent delivery module LC-10ATvp Shimadzu Liquid chromatograph pump equipped with 20 μl loop and model SPD M10Avp Shimadzu UV/VIS diode array detector. Separation was carried out on a Phenomenex, (250 x 4.6 mm) Luna 5μ C-18 (2) 100A column. The chromatographic analysis was performed at ambient temperature. The mobile phase consisted of the mixture of methanol, water and acetonitrile (44:36:20). The prepared mobile phase was filtered through ultipore N 66 nylon, 6,6 membrane (0.45 μm) filter and ultrasonically degassed prior to use. The detection wavelength was set 248.0 nm and the peak area was recorded using chromatographic data system. The flow rate was optimized to1.0 ml/min in the runtime of 15.0 minutes. The entire system suitability parameters capacity factor, plate number, tailing factor and retention time was optimized by freshly prepared standard solution of 10.0 μg/ml (Table 1).

Table 1: Separation Variable for Ezetimibe

Variable	Condition
Column	
Dimension	250 mm x 4.60 mm
Particle size	5 μm
Bonded phase	Octadecylsilane (C18)
Mobile Phase	
Methanol : Water : Acetonitrile	44 : 36 : 20
Diluent Flow rate	Methanol : Water (50 : 50) 1.5 ml/min
Temperature	Ambient
Sample size	20 μl

Table 1: Contd.,

Detection wavelength	248 nm
Retention time	10.6 ± 0.5

Method Development

To develop a suitable method accurately weighed 10.0 mg of ezetimibe working standard was transferred into 50 ml volumetric flask, dissolved in 5.0 ml of mobile phase than volume was made up to 50.0 ml with mobile phase to get concentration of solution 200 µg/ml (Stock-A). The 12.5 ml volume of stock-A was taken and diluted up to 25.0 ml to get concentration of 100 µg/ml (Stock-B). Further dilution were made from stock-B to get a series of concentration 10, 20, 30, 40, and 50 µg/ml and before the injection the solution was filter through 0.45 micron HPLC filter and subjected for analysis. Peak area under the curve of mixed standard were observed and plotted against respective concentration.

Forced Degradation

Alkaline Degradation: Accurately weighted about 50 mg ezetimibe was dissolved in 25 ml methanol and volume was made up to 50 ml with 0.2 N NaOH solution. It was kept on water-bath and temperature was maintained at 60^0C. Aliquots of sample were withdrawn at 0, 5, 15, 30 min after addition of sodium hydroxide solution. Withdrawn samples were neutralized with 0.1 N HCl and diluted with diluent to get concentration of 25 µg/ml ezetimibes sample dilutions were analysed and degradation pattern was observed. Prior to degradation analysis, three replicates of standard solution of 25 µg/ml ezetimibe were analysed to compare the degradation results.

Acidic Degradation: Accurately weighed about 50 mg ezetimibe was dissolved in 25 ml methanol and volume was made upto 50 ml with 2 N HCl solution. It was kept of water bath and temperature was maintained at 60^0C. Aliquots of sample were withdrawn at 0, 5, 15, 30, 60, 90 and 120 min after addition of hydrochloric acid solution. Withdrawn samples were neutralized with 1 N NaOH and diluted with diluent to get concentration of 25µg/ml ezetimibe. Dilutions were analysed and degradation pattern was observed. Prior to degradation analysis, three replicates of standard drug solution of 25 µg/ml ezetimibe were analysed to compare the degradation results.

RESULTS AND DISCUSSIONS

Method Development

The LC Method for estimation of ezetimibe was extensively validated as per ICH guideline linearity, range, stability, accuracy and precision (repeatability and intermediate precision). Different system suitability parameters were observed is retention times, capacity factor, tailing factor and plate number (Table 2).

Table 2: System Suitability Parameters

Parameters	Values, SD, RSD
Number of theoretical plates*	10735.67, 167.32, 0.0156
Tailing factor*	1.058, 0.023, 0.0217
HETP (mm)*	0.0228, 0.0012, 0.0526
RT (min)*	10.6 ± 0.5, 0.163, 0.0149

*Mean of six replicates

For linearity, series of dilutions were prepared and response ratios were determined respectively. The range was determined by preparing a series of dilutions from 80 % to 120 % of test concentration in six replicate. For the stability of the drugs, solutions were stored at room temperature and analysed on HPLC at different time intervals. Accuracy of

methods was determined by recovery studies in which known amounts of standard drug were added to the previously analysed tablet sample and mixture were analysed by the proposed method. Precision was studied for repeatability and intermediate precision (days, and analysts) (Table 3).

Table 3: Validation Parameters

Parameter	Values; SD, RSD
Linearity Response ratio	1-32 µg/ml29766.68, 6.2738, 0.0002
Range	2-12 µg/ml, 43.81, 0.0001
Accuracy	100.006, 0.082, 0.0008
Precision Repeatability	99.88, 0.744, 0.0074
Intermediate precision Day to Day	99.95, 0.083, 0.0008
Analyst to analyst	100.0, 0.297, 0.003
Specificity	Area of EZE peak decreased as degradent peaks arised on hydrolytic degradation

Degradation Profiling

Alkaline Conditions: Alkaline degradation of EZE was performed in 50% methanolic 0.1 NaOH at 60°C, as it is insouble in sodium hydroxide solution. It is degraded into five degradants and completely degraded till 30 min.

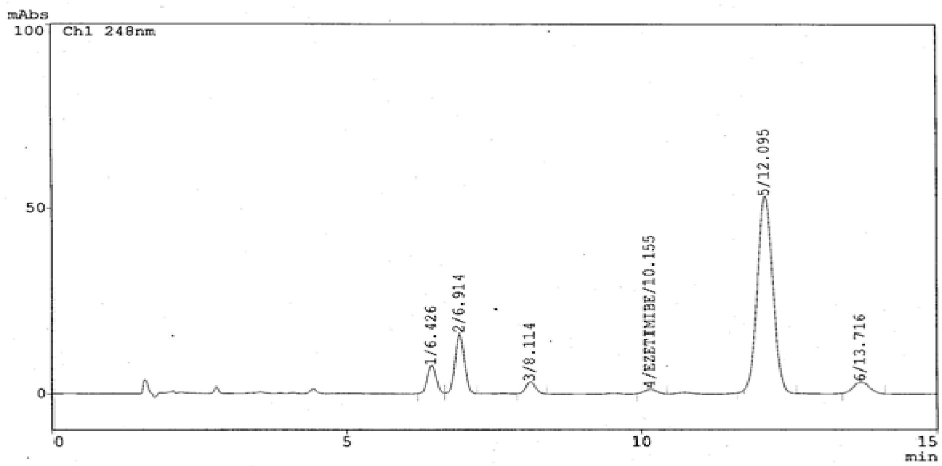


Figure 2: Chromatogram of Ezetimibe In 50% Methanolic 0.1 N Naoh at 15 Min

Degradatnts of RT 6.4, 6.9 and 12.0 min appear in 0 min sample chromatogram and degradant of RT 12.0 min is the primary degradant. Degradants of RT 8.1 and 13.7 min appear in 5 and 15 min sample chromatogram respectively (Figure 2). Thus, EZE is completely degraded into five degradants within 30 min in 50% methanolic 0.1 N NaOH at 60°C (Figure 2 and Table 4).



Figure 3: Hydrolytic Degradation Profile of EZE In 50% Methanolic 0.1 N Naoh at 60° C.

Acidic Condition: Acidic degradation of EZE was performed in 50% methanolic 0.1N HCl at 60^0C, as it is insoluble in hydrochloric acid. It is degraded upto 71.3% into three degradants till 120 min. All degradants appear in first analytical sample (Figure 4 and Figure 5). The concentration of EZE decreases as time passes but concentration of degradants vary. Thus, EZE is degraded upto 71.3% into three degradants till 120 min in 50% methanolic 0.1N HCl at 60^0 C (Table 4).

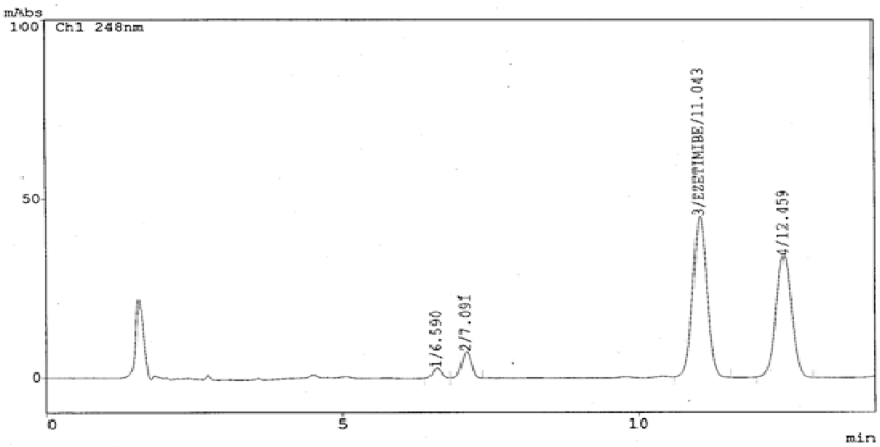


Figure 4: Chromatogram of Ezetimibe In 50% Methanolic 0.1 N Hcl at 15 Min

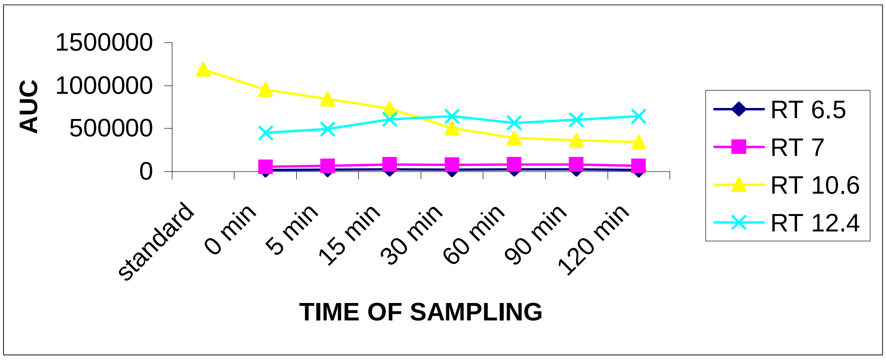


Figure 5: Hydrolytic Degradation Profile of EZE In 50% Methanolic 0.1 N Hcl at 60° C

Table 4: Remaining % of Ezetimibe during Hydrolytic Degradation

Time Sample Withdrawal	In 50% Methanolic HCl at 60°C	In 50% Methanolic NaOH at 60°C
Standard	100	100
0 Min	80.1	15.38
5 Min	70.8	5.05
15 Min	61.6	2.25
30 Min	42.3	0.0
60 Min	32.7	-
90 Min	30.4	-
120 Min	28.7	-

CONCLUSIONS

Drug was faster degraded in alkaline conditions than acidic conditions. The validated RP-HPLC method for ezetimibe may be applied for assay, stability-indicating assay, dissolution studies, as well as routine analysis in pharmaceutical industries.

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